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Feed gas humidity: small concentrations – large effects

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Abstract: The effect of feed gas humidity was investigated on an atmospheric pressure argon plasma jet, on plasma treated cell growth medium (RPMI) and finally on human skin cells. Already small humidity concentrations below 1000 ppm have a strong influence on the jet's emission signal, gas and liquid phase H₂O₂ production as well as on the cell viability. A correlation between H₂O₂ generated in the liquid and cell viability was obtained.

Keywords: humidity, atmospheric pressure argon plasma jet, plasma medicine, H₂O₂, HaCaT

1. Introduction

The present work shows answers for the relevance of unwanted – or intended – feed gas humidity in plasma medical experiments and their comparatively large relevance with respect to ambient humidity. Research in plasma medicine does not only require a full understanding of the generated plasma but also a comprehension of liquid phase processes induced by the plasma. This is since plasma treated eukaryotic cells are normally covered by some sort of liquid. Thus, reactive species generated inside this plasma treated liquid interact with the cells and trigger complex reaction cascades. By applying feed gas humidity inside an atmospheric pressure argon plasma jet (kinpen 09) as variable parameter the effects on the plasma, on the cell growth medium liquid (RPMI) and finally on human skin cells (HaCaT) were studied [1, 2].

2. Feed gas humidity affects the plasma

The plasma was investigated by using optical emission spectroscopy (OES) as well as ultra-violet (UV) and infrared (IR) absorption spectroscopic techniques. By means of axial resolved OES the strong influence of feed gas humidity on the emission of excited hydroxyl (OH), nitrogen molecules (N₂), atomic oxygen (O) and argon (Ar) is depicted in figure 1. With increasing humidity concentration in the feed gas the emission of argon and oxygen lines as well as the band emission of nitrogen decreases. Two effects are responsible for this finding. Firstly, with increasing concentration of water molecules (H₂O) in the feed gas quenching of excited states becomes more likely. Secondly, it is known, that the admixture of molecular gases leads to a reduction of electron temperature and hence to a decrease in the excitation rate of the investigated species. The emission of OH behaves differently to the other species. For small water admixtures an increase of OH emission is observed up to a humidity concentration level of about 400 parts per million (ppm). Presumably, the OH density increases with increasing H₂O admixture which leads to an increased emission sig-

nal. However, when increasing the humidity concentration beyond 400 ppm quenching of excited OH as well as the reduced electron temperature reduce the excited state density and therefore the emission signal.

Besides the OES results, measurements of the ozone production rate and on gas phase hydrogen peroxide (H₂O₂) concentration were performed by means of UV absorption and Fourier transformed infrared (FTIR) spectroscopy, respectively. An increase of feed gas humidity results in a decreased ozone production rate. On the contrary, the H₂O₂ concentration produced by the plasma jet increases almost linearly with feed gas humidity increase.

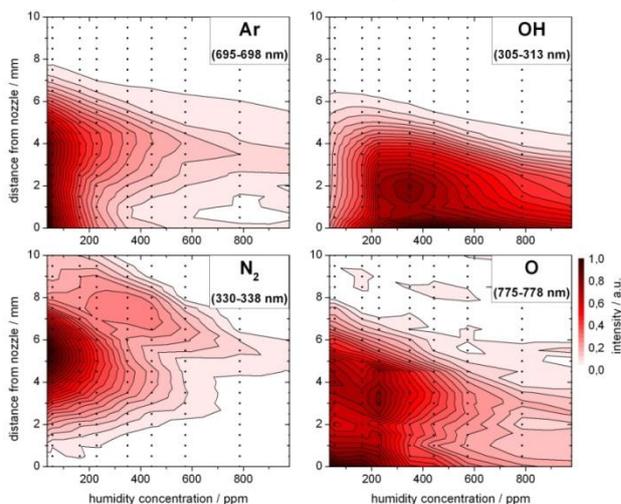


Fig. 1: Integrated emission signals originating from Ar, OH, N₂ and O in dependence on the axial distance and feed gas humidity concentration. The dots in the contour plot give the position and humidity concentration where the measurements were conducted. The intermediate values were obtained by linear interpolation.

3. Feed gas humidity versus ambient humidity

OES was also used to indicate whether feed gas humidity has a larger effect on the OH emission than ambient air humidity. When the jet was operated with dry feed gas in a humidified atmosphere (20500 ppm) the axial OH in-



tensity increases with distance to the nozzle. This is due to the fact that water molecules diffuse into the effluent and dissociate. As a result OH molecules form and become excited by the effluent plasma. Since excitation is limited in axial direction the emission profile forms a maximum. Identically to nitrogen emission, the highest emission signal was found at a distance of 5.5 mm.

On the contrary, when the jet is operated with humidified feed gas (490 ppm) in a dry atmosphere the OH emission signal decreases steadily in axial direction and no maximum evolves. Particularly interesting is the fact, that although the humidity concentration in this case is by a factor of 42 lower than for the case of wet shielding gas, a higher OH emission signal is detected (for $z < 5$ mm). In conclusion, ambient air humidity influences the plasma effluent and must be considered especially for applications that require stable plasma conditions. Indeed, much more important than controlling the ambient humidity is to consider the feed gas humidity since it affects the plasma substantially more.

4. Feed gas humidity affects liquid cell growth media

In order to analyze the effect of feed gas humidity on cell growth medium (RPMI) the H_2O_2 concentration generated in the plasma treated liquid was analyzed using Merckoquant stripes and the Amplex red chemical assay. The results are shown in figure 2. The results of both utilized methods show a remarkable agreement and indicate a linear increase of H_2O_2 concentration in the liquid with increasing feed gas humidity. For the highest investigated humidity setting a H_2O_2 concentration of almost $4 \text{ mg}\cdot\text{l}^{-1}$ has been measured after 40 s plasma exposure. Under dry gas condition still a H_2O_2 concentration of around $0.8 \text{ mg}\cdot\text{l}^{-1}$ has been measured. For both humidity settings the H_2O_2 production rate is calculated assuming a linear increase of the H_2O_2 concentration with treatment time. A production rate of $1.8 \times 10^{-3} \text{ g}\cdot\text{h}^{-1}$ for the highest humidity setting and $2.0 \times 10^{-4} \text{ g}\cdot\text{h}^{-1}$ for the driest feed gas condition is obtained. A similar value of $3 \times 10^{-4} \text{ g}\cdot\text{h}^{-1}$ was obtained by Ikawa et al. using a plasma jet driven in dry helium [3].

5. Feed gas humidity affects human skin cells

The viability of adherent HaCaT cells was determined using the conversion of resazurin by metabolic active cells into the highly fluorescent resorufin. The details of this assay are given in [4]. The cells were cultivated in the same medium that was used for the H_2O_2 measurements. After 40 s plasma treatment of 5 ml RPMI solution under the equivalent conditions as for the H_2O_2 measurements, the plasma treated medium was immediately transferred into one row of wells of the cell culture plate and serially diluted with complete medium immediately (six parallel wells per treatment time) to obtain different treatment time equivalents. Analogue to the procedure described in

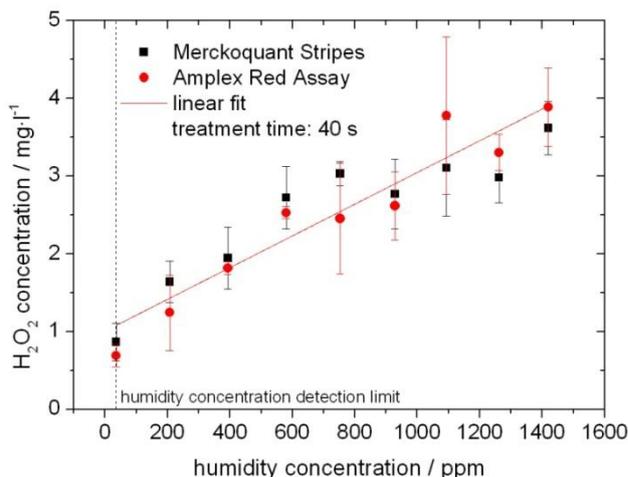


Fig. 2: H_2O_2 concentration in plasma treated liquid increases with feed gas humidity. Merckoquant stripes and the Amplex Red chemical assay were utilized for the H_2O_2 detection.

[4] the cells were cultivated and their fluorescence intensity was analyzed by a Tecan M200 multiplate reader. As a measure of viability the fifty percent inhibition value was determined for every investigated feed gas humidity level.

The results are depicted in figure 3. With increasing feed gas humidity the viability of the human skin cells decrease. Since indirect treatment of the cells was applied (cells were not directly exposed to the plasma) only long living species in the liquid can lead to the observed cell reaction. Considering the H_2O_2 increase in the liquid it is reasonable to assume that H_2O_2 is responsible for the observed effect on the cells.

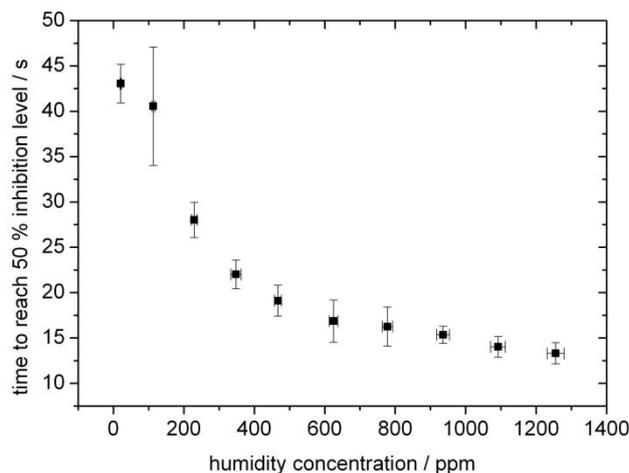


Fig. 3: Cell viability in dependence on the feed gas humidity concentration. The cells were treated indirectly

Considering the findings of the H_2O_2 concentration measurements of section 4 correlation between cell viability and H_2O_2 concentration becomes obvious. This means when the H_2O_2 concentration is low, the cell viability is high. With increasing H_2O_2 concentration a decrease in cell viability is also observed. In conclusion, by applying

the plasma source in our eukaryotic cell model the cells were exposed to ROS and, hence, to oxidative stress, which exceed the cells' capacity for detoxification and repair. Although H₂O₂ plays an important role in the oxidative stress that affects the cell viability, other reactive species such as OH or O₂⁻ might also be involved in the observed effects. Cell viability could be influenced by mechanical stress or electric fields derived from the plasma source. Since indirect plasma treatment was applied, these and other stress factors such as UV radiation were excluded for our experimental conditions.

6. Conclusion

In the present paper it is described that feed gas humidity not only affects excited species (OH, N₂, O, Ar) and reactive ground state molecules like O₃ and H₂O₂ in the plasma, but also leads to a linear H₂O₂ increase in plasma treated cell growth medium. When human skin cells get challenged with this medium a strong correlation between the cell viability and the H₂O₂ concentration is revealed. Furthermore, it was shown by means of OES on OH emission that feed gas humidity is more crucial than ambient humidity. However, when dry feed gas is applied ambient humidity becomes relevant again.

Acknowledgements

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References

- [1] J. Winter, K. Wende, K. Masur, S. Iseni, M. Dünbier, M. U. Hammer, H. Tresp, K.-D. Weltmann, S. Reuter, J. Phys. D: Appl. Phys., in press (2013)
- [2] K.-D. Weltmann, E. Kindel, T. von Woedtke, M. Hähnel, M. Stieber, R. Brandenburg, Appl. Chem., 82, 1223-37 (2010)
- [3] S. Ikawa, K. Kitano, S. Hamaguchi, Plasma Process. Polym., 7, 33-42 (2010)
- [4] S. Reuter, H. Tresp, K. Wende, M.U. Hammer, J. Winter, K. Masur, A. Schmidt-Bleker, K.-D. Weltmann, IEEE Trans. Plasma Sci., 40, 2986-93 (2012)